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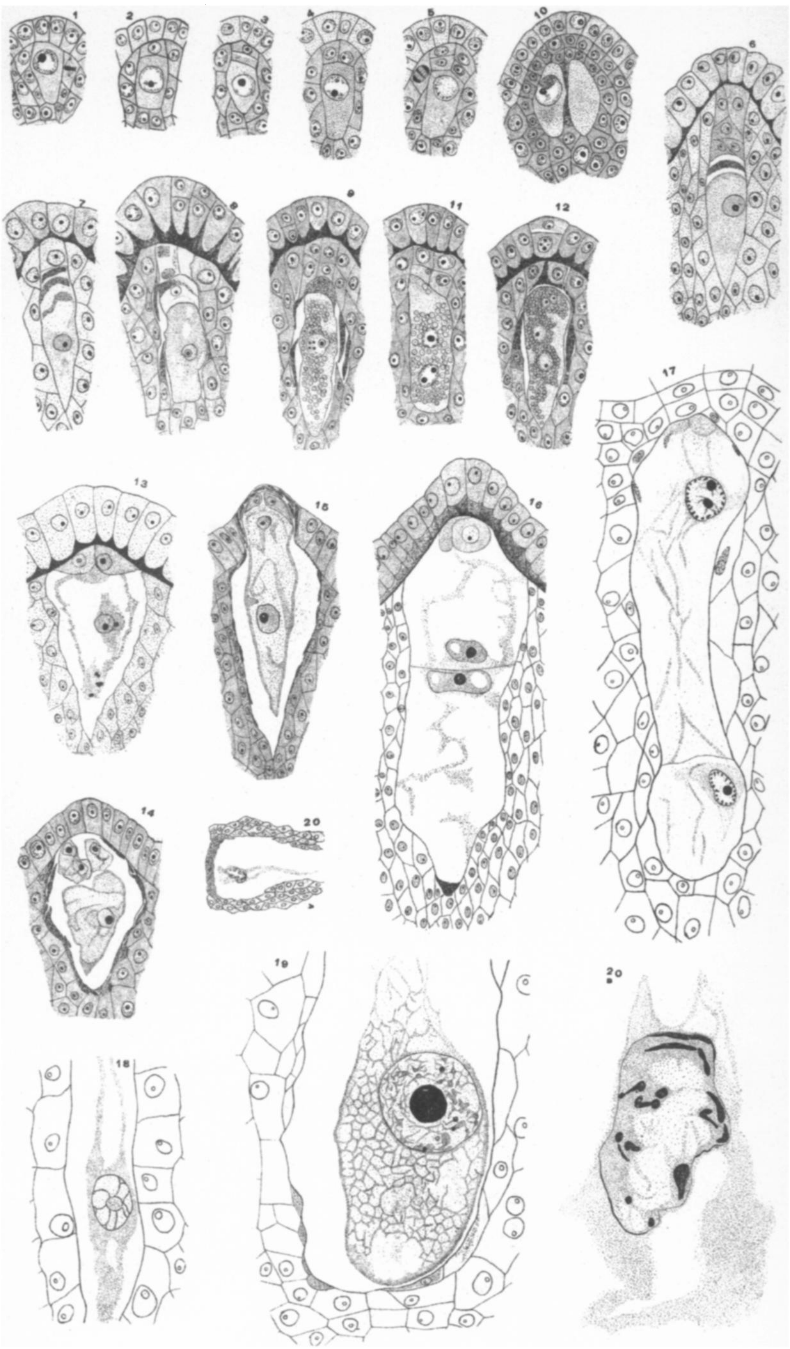

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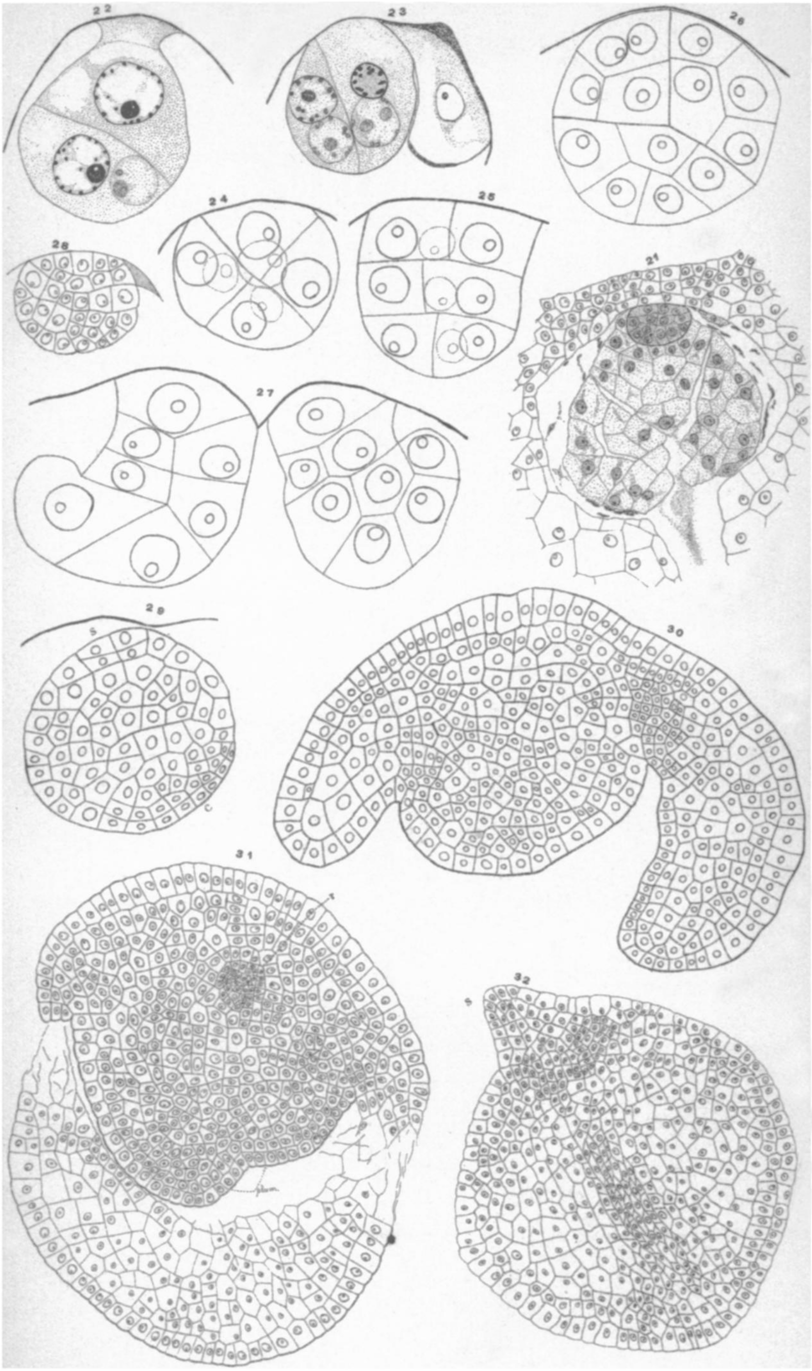
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Development of the Embryo-sac and Embryo of Castalia odorata and Nymphaea advena*

BY MELVILLE THURSTON COOK

(WITH PLATES 12 AND 13)

The systematic position of the Nymphaeaceae has long been an unsettled question, since the family presents some characters of both monocotyledons and dicotyledons. The purpose of this study was to reach some satisfactory conclusion as a result of observation on the development of the embryo-sac and the embryo. *Castalia odorata* Dryand, and *Nymphaea advena* Soland, were selected and the study has shown that they differ in very few and minor points in their development. Since beginning these investigations, Lyon has published "Observations on Embryogeny of Nelumbo" (9) in which he reaches the conclusion that it is a true monocotyledon in which the single cotyledon bifurcates, thus giving rise to the two fleshy bodies.

The material was killed and fixed in Flemming's osmic-chromo-acetic solution and in chromo-acetic. It was then washed, passed through alcohol, imbedded in paraffine and cut with Minot microtome. The stains used were haematoxylin and iron alum, safranin and gentian violet, and cyanin and erythrosin. The first and second combinations were especially successful. The safranin and gentian violet was especially good for demonstrating the antipodals. A large number of slides were prepared and most of the points figured were observed many times.

DEVELOPMENT OF THE MEGASPORE AND EMBRYO-SAC

The archesporial cell develops from the hypodermis and is easily distinguished from the surrounding cells. In one instance two archesporial cells were observed. The archesporial cell enlarges (*fig. 1*) and the first tapetal cell is formed by a transverse division (*fig. 2*). Other tapetal cells are formed in a very irregular manner as a result of the division of the first. Two tapetal cells may be formed by a periclinal wall (*f. 3*) or by an anticlinal wall (*f. 4*) or three may be formed by the formation first of an anticlinal wall followed by a periclinal wall in one cell only, or four may be formed by two periclinal walls followed by one anticlinal in one cell only, or by one anticlinal wall followed by two periclinal walls (*f. 5*).

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* Contributions from the Botanical Laboratory, Ohio State University.

The formation of the potential and the functional megaspores was not observed but many cases of the growth of the functional megaspore and degeneration of the potential megaspores were observed (*f. 6–9*). In one case two well-developed functional megaspores were observed lying side by side (*f. 10*).

The embryo-sac is perfectly straight and develops rapidly, enlarging principally in the direction of its longer axis, the greater growth being towards the micropyle at the expense of the tapetal cells (*f. 11–13*). An abundance of starch usually appears in the developed megaspore and persists throughout the two- and four-celled stages (*f. 9, 11, 12*). The antipodal cells are very small and disappear about the time of the conjugation of the polar nuclei (*f. 13*). In only one instance were they observed to persist until the beginning of the formation of the endosperm.

With the formation of the endosperm nucleus the egg apparatus becomes very distinct (*f. 14, 15*). The synergids are quite apparent and at least one of them persists for a very long time (*f. 23, 28*).

Shortly after the conjugation of the polar nuclei (*f. 13, 14, 15*) and fertilization of the egg (*f. 16*) the endosperm nucleus divides and a very delicate but definite wall is formed across the sac (*f. 16, 17*) similar to that described by Schaffner for *Sagittaria* (**12**).

The upper nucleus thus formed moves toward the embryo and rapidly forms the endosperm (*f. 17* and *text fig. 1*). This end of the sac enlarges rapidly and becomes filled with endosperm (*f. 21*) which persists until the embryo is fully formed but the cell walls gradually become thinner and the protoplasm gradually disappears as the embryo approaches maturity.

The other cell formed by the division of the endosperm nucleus grows towards the chalazal end of the ovule, forming a tube by the absorption of the nucellus and leaving in its trail a long string of protoplasm reaching back to the endosperm which never enters this tube (*f. 17, 18, 19* and *text fig. 1*). The formation of this tube is in the axis of the ovule, where the nucellus cells are slightly elongated and have very thin walls. These cells are absorbed very readily, the fragments along the sides of the tube usually being very few and very small. The nucleus reaches the chalazal end of the ovule about the time the embryo is in quadrant stage (*text fig. 1*). Upon reaching the chalazal end of the ovule the end of the tube enlarges, the cell making a corresponding enlargement and then degenerating (*f. 20, a, b*). This process is complete when the embryo and endosperm are in the stage represented by *f. 21*.

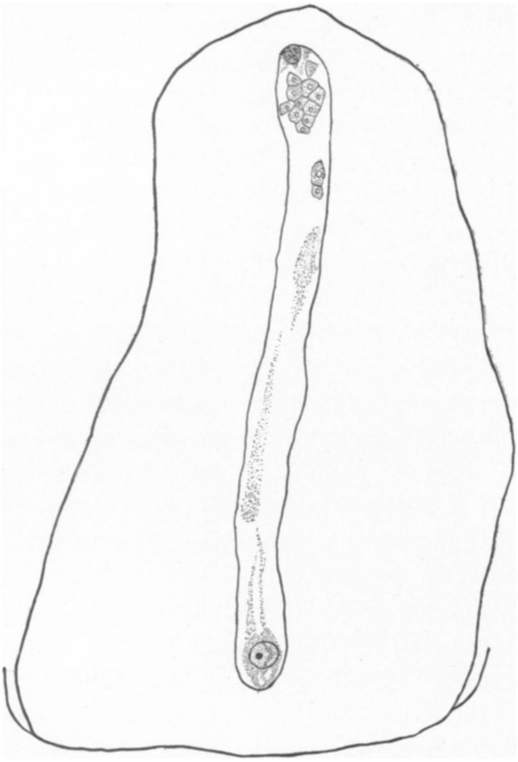

FIG. 1. Tube formed by lower endosperm nucleus. Three-celled embryo and endosperm in upper end of tube, lower endosperm nucleus in lower end of tube. Fig. 19 drawn from same section.

Hegelmaier, in his "Untersuchungen über die Morphologie der Dikotyledonen-Endosperms" (**7**), describes four types of endosperm development which he designates as: (1) "Allseitig-peripherische" (*e. g., Adonis, Caltha, Cotoneaster, Malva, Hibiscus*). (2) "Peripherisch-simultanen" (*e. g., Bocconia, Scabiosa,, Euphorbia*). (3) "Einseitig peripherischen" (*e. g., Trigonella, Phaseolus, Fagopyrum*). (4) "Endogene" (*e.g., Eranthis*). In the Nymphaeaceae we apparently have a type intermediate between the third and fourth with greater resemblance to the third type, but differing from it in the appearance and activity of the lower endosperm nucleus.

The physiological significance of this nucleus presents a very interesting problem which should be considered in connection with the function of the antipodals.

In the case of the Ranunculaceae as described by Coulter (**1**) the very large antipodals indicate some very important function which is emphasized by Campbell's study of *Sparganium simplex* (**5**) in which he describes a very great increase in the number of antipodal cells. Lloyd's researches on *Vaillantia hispida* (**10**) seem to throw some light on this problem. He describes the behavior of the antipodals, the third and lowest of which form a haustorium which projects back into the disintegrating megaspores and apparently bearing a nutritive relation to the embryo-sac. Guignard in his recent paper on *Naias major* (**6**) describes two of the antipodals degenerating and the upper one enlarging long after the fertilization of the öosphere.

Chamberlain in his paper on *Aster Novae-Angliae* (**3**) describes and figures a peculiar enlargement of the lower antipodal and believes that one of the nuclei represents an oösphere, thus emphasizing Strasburger's view that the antipodal region is homologous with the prothallium of the Gymnosperms.

Whatever may be the physiological or morphological significance of the antipodals in the plants to which we have just referred and whatever it may have been in the past we find many plants to which Strasburger's view cannot be applied.

In many cases the antipodals are very small and without function but we frequently find other structures which are apparently performing a similar function to that described by Lloyd for *Vaillantia hispida* (**10**). In *Lilium Philadelphicum* (**2**) Coulter describes an enlargement of the sac with the three disorganizing antipodals in a small caecum-like structure. Riddle (**11**) in her studies on *Alyssum macrocarpum* describes a decided enlargement of the sac, and an increase in the endosperm with a very pronounced accumulation of endosperm cells in the antipodal region, usually obscuring the antipodals which are very small and apparently functionless.

In Schaffner's studies of *Sagittaria variabilis* (**12**) we have a condition very nearly that found in the Nymphaeaceae. The endosperm nucleus divides and a wall is formed across the sac; the lower part of the sac does not enlarge greatly as in the Nymphaeaceae and the antipodals persist but the nuclei resemble very closely those I have described for the Nymphaeaceae.

We find further evidence on this point in the papers on *Potamogeton* by Wiegand (**15**) and by Holferty (**8**). Wiegand describes for *P. foliosus* (**15**) a sac of seven nuclei, three forming the egg apparatus and three the antipodals and one very large nucleus which he calls the fourth antipodal and states that a little later it divides into two and that a wall forms across the sac at this point, separating the two nuclei thus formed. He furthermore states that the upper of these two new nuclei divides forming the endosperm, the lower remaining undivided. Holferty describes *P. natans* (**8**) as forming the normal eight-celled sac and the polar nuclei as fusing in the normal manner.

From these two papers it seems possible that Wiegand failed to observe the conjugation of the polar nuclei and that the division of the fourth large antipodal was really a division of the endosperm nucleus as in *Sagittaria* and the Nymphaeaceae.

It seems unreasonable to suppose that the antipodals are without physiological significance in those plants where they are prominent. In the Ranunculaceae, in *Sparganium*, and in *Vaillantia* the antipodals appear to furnish nourishment for the embryo. The peculiar haustorial development of the antipodal of *Vaillantia*, the enlargement of the lower antipodal in *Aster*, the accumulation of endosperm in the antipodal region of *Alyssum*, and the large lower nucleus formed by a division of the endosperm nucleus in *Sagittaria* and the Nymphaeaceae show a resemblance which I believe indicates similar physiological functions. The mere enlargement of the sac of *Lilium* and many other plants may accomplish the same result by absorption of the surrounding cells of the nucellus.

A very pronounced sclerification was developed in the inner part of the epidermal cells just before maturity of the embryo-sac (*f. 6–9, 11–13, 16*). These cells separated readily from the underlying cells of the nucellus and from each other; often the microtome knife would tear them loose and pressure upon the cover-glass of a fresh preparation was usually sufficient to separate them. Although fertilization was observed but once (*f. 16*) and then the path of the pollen tube could not be traced, I am inclined to believe that it occurs in the usual manner.

DEVELOPMENT OF THE EMBRYO

The embryo becomes very prominent (*f. 16*) and divides by a transverse division which is usually oblique (*f. 22, 23*). This is followed by the formation of a quadrant (*f. 23*) and an octant (*f. 24*), the embryo retaining its spherical form. In one instance a distinct twelve-celled embryo (*f. 25*) was observed. The formation of the octant is not always regular and the divisions following this are very irregular, especially in *N. advena*, but the embryo retains its spherical condition until fully half developed and becomes free in the sac (*f. 26, 29*). The free spherical condition corresponds to the condition observed in *Nelumbo* by Lyon (9).

In one case polyembryony (*f. 27*) was observed and was apparently due to the splitting of a very young embryo, since the embryo may typically be said to have two or four basal cells, while in one case of polyembryony each embryo showed one distinct basal cell.

When well advanced there is an increased activity in the division of the cells on the side of the embryo farthest from the micropyle (*f. 29, c*). This is the first appearance of the cotyledon the growth being forward and lateral, the edges being slightly curved so as to form a sort of elongate tray with the main spherical part of the embryo placed at one end (*f. 30, 31*).

A few cells next to the micropyle, probably the original basal cells, show increased activity shortly after the first appearance of the cotyledon growth and eventually forms the suspensor which projects towards the micropyle as a short cone-like organ, but does not become attached to the walls of the embryo-sac (*f. 29, s, 32, s*) and does not appear to be of any great value.

The cotyledon was not observed to bifurcate (*text fig. 2*), as Lyon describes for *Nelumbo* (9), although embryos older than those figured were examined. Soon after the appearance of the suspensor there is an increased activity of the remaining spherical part of the embryo, resulting in the formation of a plumule (*f. 31, plum.*). This agrees with *Nelumbo* (9) as described by Lyon. The primary root is formed by an increased activity of cells between the suspensor and plumule (*f. 31, r*) and also agrees with *Nelumbo* as described by Lyon.

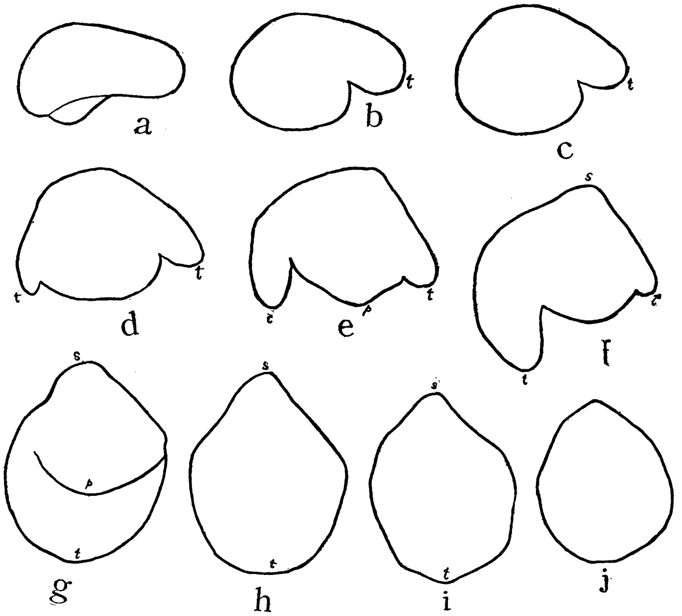

FIG. 2. Outline drawings from series of sections of single embryo of *N. advena* showing relation of parts. *s*, suspensor; *p*, plumule; *t*, cotyledon.

The embryo in its development cannot be said to show a marked resemblance to any of the monocotyledonous plants to which we might suppose it to be related. Aside from the suspensor it compares fairly well with *Lilium* and *Erythronium*. However, the development of the embryo-sac bears a striking resemblance to *Potamogeton*, *Naias*, and *Sagittaria*. The formation of tapetal cells and of megaspores is very similar to that described by Holferty for *Potamogeton* (8) and Campbell for *Naias* (4). The first division of the definitive nucleus and the formation of the wall across the sac is the same as observed by Schaffner in *Sagittaria* (12).

Lyon concludes that the Nymphaeaceae should be classified among the Monocotyledons in the series Helobiae (Naiadales). Van Tieghem in "Elements de Botanique" (14) makes a special class including the Nymphaeaceae and Gramineae between the Monocotyledons and the Dicotyledons which he calls "Liorhizes Dicotylees." Taking into consideration the general anatomy and habits of the plants, and the fact that the formation of a lower endosperm nucleus is similar to *Sagittaria* (12), it appears that the Nymphaeaceae should be placed in or near the order Naiadales.

SUMMARY

1. The archesporial cell is hypodermal.
2. The formation of the tapetal cells is irregular.
3. The formation of the megaspores is after the usual manner.
4. The embryo-sac usually contains starch in the one-, two-, and four-celled stages.
5. The formation of the eight-celled embryo-sac is in the usual manner.
6. At least one of the synergids persists for a very long period.
7. The antipodals are very small and disappear early.
8. The endosperm nucleus divides and a wall is formed across the embryo-sac between the two nuclei. The upper forms true endosperm, the lower a tube extending to the chalazal end of the ovule for absorbing the cells of the nucellus.
9. The embryo is at first spherical and later forms a single cotyledon.
10. The suspensor is late in forming and apparently without function.
11. The family Nymphaeaceae should be placed in or near the order Naiadales.

The writer wishes to express his thanks to Professor J. M.

Coulter and Dr. Charles J. Chamberlain, of the University of Chicago, for valuable suggestions in the first part of the investigation, and to Professor J. H. Schaffner, of the Ohio State University, for very valuable assistance in the later studies.

LITERATURE

Only those papers are cited to which reference has been made.

1. **Coulter, J. M.** Contribution to the Life History of Ranunculus. Bot. Gaz. **25** : 73–88. 1898.
2. **Coulter, J. M., et al.** Contributions to the Life History of *Lilium Philadelphicum*. Bot. Gaz. **23** : 412–452. 1897.
3. **Chamberlain, C. J.** The Embryo-sac of *Aster Novae-Angliae*. Bot. Gaz. **20** : 205–212. 1895.
4. **Campbell, D. H.** A morphological Study of *Naias* and *Zannichellia*. Proc. Cal. Acad. Sci. **1** : 1–61. 1897.
5. **Campbell, D. H.** Studies on the Flower and Embryo of *Sparganium*. Proc. Cal. Acad. Sci. **1** : 293–328. 1899.
6. **Guignard, L.** La double fecondation dans le *Naias major*. Jour. de Bot. 15. 1900.
7. **Hegelmaier, Dr.** Untersuchungen über die Morphologie der Dikotyledonen-Endosperms. Nova Acta d. K. L. C. D. Akad. d Naturforcher. 49. 1885.
8. **Holferty, G. M.** Ovules and Embryo of *Potamogeton natans*. Bot. Gaz. **31** : 339–346. 1901.
9. **Lyon, H. L.** Observations of Embryogeny of *Nelumbo*. Minn. Bot. Studies, **2** : 1901.
10. **Lloyd, F. E.** Comparative Embryology of the Rubiaceae. Mem. Torrey Club, **8** : 1–26. 1899.
11. **Riddle, L. C.** The Embryology of *Alyssum*. Bot. Gaz. **26** : 314–324. 1898.
12. **Schaffner, J. H.** The Life History of *Sagittaria variabilis*. Bot. Gaz. **23** : 252–273. 1897.
13. **Schaffner, J. H.** A Contribution to the Life History and Cytology of *Erythronium*. Bot. Gaz. **31** : 369–387. 1901.
14. **Van Tieghen, Ph.** Elements de Botanique. 1898.
15. **Weigand, K. M.** The Development of the Embryo-sac in some Monocotyledonous Plants. Bot. Gaz. **30** : 25–47. 1900.

Explanation of Plates

PLATE 12

Castalia odorata (except Fig. 16 which is *C. Zanzibariensis*).

FIG. 1. One archesporial cell.

FIG. 2. One archesporial cell and one tapetal cell formed by a periclinal wall.

FIG. 3. Archesporial cell and two tapetal cells formed by periclinal wall.

FIG. 4. Archesporial cell and two tapetal cells formed by anticlinal wall.

FIG. 5. Archesporial cell and four tapetal cells.

FIG. 6. Functional megaspore and three dissolving megaspores and two tapetal cells

FIG. 7. Functional megaspore and dissolving megaspores and tapetal cells.

FIG. 8. Functional megaspore and dissolving macrospores and tapetal cells.

FIG. 9. Functional megaspore and dissolving megaspore and tapetal cells. Starch in functional megaspores.

FIG. 10. Two functional megaspores. (Missing nucleus was in next section.)

FIG. 11. Two-celled embryo-sac containing starch.

FIG. 12. Four-celled embryo-sac containing starch.

FIG. 13. Eight-celled embryo-sac Conjugating polar nuclei. Degenerating antipodals.

FIGS. 14, 15. Mature embryo-sac with egg apparatus and endosperm nuclei.

FIG. 16. Fertilized egg and pollen tube. First division of the endosperm nuclei. The two nuclei separated by a wall.

FIG. 17. Separation of the two nuclei after division of the definitive nucleus. Fertilized egg and one synergid.

FIG. 18. Lower nucleus formed by division of definitive nucleus about one-third way down ovule.

FIG. 19. Lower nucleus (from text figure 1).

FIG. 20*a*. Lower nucleus in the enlarged end of the tube. Nucleus degenerating.

FIG. 20*b*. Same as 27*a*.

PLATE 13

Nymphaea advena (except Figs. 21–24, which are *C. odorata*).

FIG. 21. Upper end of tube enlarged and containing embryo and endosperm. Age corresponds to Fig. 20.

FIG. 22. Embryo of three cells. One synergid.

FIG. 23. Embryo of four cells. One synergid.

FIG. 24. Embryo of eight cells.

FIG. 25. Embryo of twelve cells. (The other three cells were found in the next section.)

FIG. 26. Spherical embryo.

FIG. 27. Double embryo.

FIG. 28. Spherical embryo. One synergid.

FIG. 29. Spherical embryo showing beginning of cotyledon (*c*) and suspensor (*s*)

FIG. 30. Embryo showing spherical part of embryo and the cotyledon.

FIG. 31. Embryo showing cotyledon, two leaves of plumule (*plum.*) and root (*r*).

FIG. 32. Section from same embryo as 31 showing suspensor (*s*).